California Medicine

Immunization Against Viral Diseases

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■ *Means are now at hand, if properly employed, to virtually eliminate clinical poliomyelitis and measles from this country. If such control is to be accomplished, more effective means are required to reach virtually all of the four million infants born each year in this country. Influenza can be suppressed, and improvements in influenza vaccine have been achieved in recent years.*

It seems likely at this time that at least several of the more important viral diseases can be controlled by utilizing antigens based on the biologic characteristics of the agent, and directed toward the reservoir of infection and the conditions favoring transmission of the infection.

The theoretical problem of the effects in man of viruses that are oncogenic in rodents and are derived from various tissue culture systems deserves serious attention. However, this consideration, that of antigenic potency, and other problems reviewed should not be allowed to subvert efforts to solve the real problems that face us, the disability and death resulting from these common infections.

EFFECTIVE CONTROL of infectious disease remains a problem of major importance. Despite the availability of numerous antigens and virtually complete control, at least in this country, of the classic plagues of mankind, infectious disease still accounts for a major portion of absenteeism in industry and schools and remains a significant cause of death.

It is the purpose of this communication to review the present status of immunization against various viral diseases. Many methods of appraising the extent of the task ahead have been employed. The excellent data on family illness accumulated by Dingle and his colleagues at Western Reserve University, data concerning the causes of school absenteeism at various ages, illnesses in industry and the many careful studies done in military populations have defined certain types of disease and their relative importance under certain conditions.

To those of us associated with hospitals it is apparent that our limited hospital resources are often stretched to the breaking point by variations in the prevalence of various communicable diseases. As noted in Table 1, infectious disease accounted for more than half of the total admissions to the Children's Division during the four months preceding the preparation of this manuscript. If one subtracts the 622 admissions to the Communicable Disease Service from this total and considers the Pediatric Service alone, 1,098 of 2,511

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TABLE 1.—*Admissions to Children's Division Los Angeles County General Hospital November 1964-February 1965*

Diagnostic Category	Numbers Admitted in Four-Month Period				
	November	December	January	February	Total
Bronchiolitis	22	31	119	184	356
Croup and LTB	43	23	13	19	98
Pneumonia	59	80	135	149	423
Communicable Disease Service	142	180	167	133	622
Misc. Infections	54	50	66	51	221
Non-Infectious	290	391	399	333	1413
Total	610	755	899	869	3133
Per Cent Infectious:	52.5	48.2	55.6	60.8	54.9

admissions or 43.7 per cent were for various infectious disease problems. Even more important, during this four-month period there was an increase of more than 800 per cent in one specific clinical disease entity, bronchiolitis, which involves young infants almost exclusively. Admissions increased from 22 in November to 184 in February, 1965. This increase has been confirmed as again associated with respiratory syncytical virus,* an agent commonly associated with this illness in infants and young children on our service as elsewhere. This illness is not simply a nuisance; five of the patients admitted for acute bronchiolitis (or over 1 per cent) died (Table 2). In passing, it is apparent that various infectious diseases were responsible, at least in part, for 49 of 72 (68 per cent) of all deaths excluding newborns on our combined Communicable Disease and Pediatric Services during this period, and for 20 of 45 (45 per cent) of deaths on the Pediatric Service alone.

While it is apparent that the majority of deaths from infectious diseases are due to bacteria, the importance of viral disease as a cause of morbidity and mortality is apparent. In addition, data on animals[24] and on infants[5] support the concept of bacterial-viral synergism as a mechanism of disease production. Careful studies to further establish the observation of Mathies[16] indicating wide seasonal variations in *H. Influenzae* meningitis mortality and the mechanism underlying the well known increased incidence of meningococcal disease in military recruits and bacterial pneumonia in young adults seen during influenza virus outbreaks, may be helpful in further defining the role, and subsequent control, of viral infection in the incidence, pathogenesis and virulence of bacterial disease.

*Virus isolations under supervision of Bernard Portnoy, M.D.

Approaches for Control

For adequate control of viral infection three approaches seem possible. The first, active immunization, appears to be the most promising at the present time. The second, an alteration of host defenses, is at least a theoretical possibility. Chemoprophylaxis, the third, is an interesting concept and recent work utilizing chlorguanide in prophylaxis of malaria indicated that effective prophylaxis for at least one year follows a single dose of this agent.[3] This suggests that effective long-term chemoprophylaxis may be a possibility with other organisms in the future. However, as a caution to hopes raised by this approach in malaria and by drugs effective against viral agents, one must recall that the most nearly ideal antibacterial agent, penicillin, has not provided the type of control we desire against even the extremely penicillin-sensitive group A streptococcus.

Nonspecific Host Factors

Even in Southern California the seasonal variation in prevalence of various communicable diseases is difficult to explain on the basis of direct influence on the parasite alone. Although it is difficult to identify individual human factors important in resistance and study them in detail, the recent recruit studies by Pierce and coworkers,[21] which have yet to be confirmed, suggest that a delay in the staging of routine immunization proce-

TABLE 2.—*Total Cases and Deaths, Children's Division Los Angeles County General Hospital November 1964-February 1965*

Diagnostic Category	Patients Admitted to Service			Mortality Per Cent
	Recovered	Died	Total Admissions	
Bronchiolitis	353	5	365	1.37
Other Respiratory	514	7	521	1.34
Communicable Disease Service	593	29	622	4.66
Misc. Infections	213	8	221	3.62
Non-Infectious	1388	25	1413	1.77
Total	3061	74	3142	2.36

dures in recruit populations was associated with a decrease of about 20 per cent in the incidence of significant respiratory disease during a ten-week training period. If confirmed, and perhaps extended by further studies of host-virus-bacterial interaction, this type of observation suggests that a technique may be at hand to evaluate specific factors in humans that may be altered by stress.

The discovery of interferon by Isaacs and Lindemann[11] offered hope that a nonspecific mechanism effective against many types of viral disease and produced by many types of host cells could be stimulated by vaccines or perhaps be supplied artificially to provide an increase in host resistance. Further studies have shown that this material is a protein produced by a wide variety of cells and in response to many types of viral infection and that it retains its activity over a wide range of pH values. It is only weakly antigenic, and thus would not be expected to be eradicated by normal host defense mechanisms. The initial hope that this interesting material would be useful in prophylaxis or therapy following stimulation by various vaccines has not been borne out by further studies with the possible exception of the transient rise following attenuated measles vaccine.[20] There seems to be little likelihood of stimulating increased production of long duration by vaccines or other techniques in the host, and commercial production of this material in the laboratory and its effective use seems most unlikely at this time. It should presently be regarded as an interesting substance illustrating one of the mechanisms of host defense against viral infection[1] and as such represents an extremely important contribution.

Active Immunizations

Vaccinia virus was demonstrated to be effective in the prevention of smallpox more than 150 years ago, and more recently several other effective vaccines were prepared either in animals or in chick embryos. The most important single discovery, the adaptation of poliovirus to tissue cultures by Enders, Weller and Robbins,[7] opened a new era in the cultivation of many viruses. This approach has led to the isolation and characterization of more than 100 viruses from the respiratory tract and a nearly equal number from the gastrointestinal tract of man alone.

Production of viruses is now possible in the absence of substantial quantities of host tissue, facilitating further purification. Equally important, the selection of virus strains from single particles for certain characteristics is also possible by altering cell types and temperature. It is now possible to characterize many viral diseases and to determine their relative importance, prevalence and extent of antigenic variation. Persistence and prevalence of circulating antibody against these agents can also be ascertained relatively easily.

As a result of both epidemiologic and laboratory investigation, it has become apparent that some types of viral infection are caused by agents of relatively stable characteristics and with long-lasting resistance to clinical reinfection. Examples of these, as suggested by Hilleman,[9] are smallpox, yellow fever, other arboviruses, measles, mumps, rubella, varicella and poliomyelitis.

It is also becoming increasingly apparent that some viruses are not associated with similar long-lasting immunity to reinfection. The respiratory virus complex, particularly some of the more recently recognized agents, appear to fit this category, with demonstrated reinfections in persons presumably immune by virtue of previous infection. The best examples of this category are the respiratory syncytial and parainfluenza viruses.[12] While, in the former group, selected attenuated strains may be most effective and offer the most reasonable approach to complete control, inactivated vaccines, particularly with adjuvants, may hold the most promise in the latter situation. It seems unrealistic to expect a selected or attenuated agent to provide more effective and durable protection than that following the natural infection with the wild agent. However, one should recall that even relatively short-lived protection, if effective, may be extremely valuable, as in the young infant against respiratory syncytial virus, or in the pregnant woman against rubella.

As in the case with other discoveries, the advent of tissue culture methods for vaccine preparation has been associated with entry into a complex field with many unknowns, still unresolved. The promises and problems in immunization against viral diseases can best be illustrated by specific examples of vaccines either now licensed or under study. The promises are apparent to all, while the problems and unknowns have involved consistency and duration of effectiveness and potency, adequate safety standards for use in man, and the presence in tissue culture systems of hitherto unrecognized latent viruses, some with oncogenic potential in animals.

TABLE 3.—*Measles and Measles Encephalitis State of California, 1955-1964 (Data from California State Department of Public Health)*

Year Reported	Measles		Measles Encephalitis		Total from Measles
	Cases	Deaths	Cases	Deaths	Deaths
1955	68,961	28	72	9	37
1956	32,741	7	52	7	14
1957	53,543	16	95	11	27
1958	36,231	7	66	5	12
1959	41,018	17	96	13	30
1960	22,648	6	68	6	12
1961	39,201	13	98	11	24
1962	28,585	9	80	5	14
1963	25,058	21	87	7	28
1964	34,451	N.A.*	83	11	(Incomplete)

*Not available.

One of the clearest illustrations of both promises and problems is that of measles virus, first isolated slightly more than a decade ago.[6] It has long been apparent to epidemiologists, and more recently to virologists, that this agent is not subject to significant antigenic variation and that naturally acquired infection is followed by life-long immunity to disease and probably to reinfection as well. The need for prophylaxis is readily apparent when one considers the severity of the illness, the frequency of bacterial complications and the occurrence of clinically apparent encephalitis in approximately 0.1 per cent of the cases. The latter is frequently followed by either motor or intellectual impairment among those who survive. Figures for California alone emphasize this need, as noted in Table 3, and it is apparent that the frequency of encephalitis, disregarding all other considerations, justifies the routine use of a preventive agent for this disease.

As with other viruses, during the attempts to prepare measles virus for vaccine development and early field trials it was discovered that at least one and perhaps several "fellow travelers" were present. These agents, known for many years as the avian-leucosis group, are viruses associated with various types of avian malignant disease and are commonly found in chickens and in the chick embryo tissue culture system used for propagation of this virus. While it is true that in the past many persons had received yellow fever vaccine contaminated with these agents without demonstrable harmful effect, these recipients were mainly adults. Since measles vaccine would be given to young children, and the young of tumor-susceptible species are less resistant than older animals, the proper decision was made to withhold vaccine licensure until assurance was gained that measles vaccine would be free of these agents.[26]

As a result of extensive trials by many investigators, both inactivated measles virus and the active attenuated Edmonston B virus were thoroughly evaluated. The latter was used alone, with gamma globulin or following one or two doses of inactivated vaccine given at monthly intervals. At present it would appear that the inactivated vaccine provides temporary resistance to disease and little protection, if any, to subclinical infection. The attenuated Edmonston B strain, when used alone, has been associated with frequent febrile reactions, and convulsions have been reported in about 2 per cent of recipients.[23] These reactions are sufficiently frequent to cause appreciable concern among parents, a concern also expressed among physicians who have recently used this strain again without gamma globulin or previous modification with inactivated vaccine.

As a result of an additional 85 passages in chick embryo tissue culture at reduced temperatures, a further attenuated virus was obtained by Schwartz.[23] This variant appears to have retained the antigenic characteristics of naturally occurring measles, the lack of infectivity to contacts of recently immunized persons and adequate antibody response characteristic of Edmonston B virus with substantially fewer febrile reactions and a lower incidence of rash when given alone.[14]

With the licensing of the further attenuated measles vaccine strain on February 7, 1965, three different vaccines are now available and at least five routines may be followed. The three vaccines are the inactivated vaccine, the Edmonston B attenuated and the further attenuated vaccine strain. While there are no known contraindications to immunization with inactivated measles vaccine, it has become apparent that the duration of immunity with the use of this vaccine alone is relatively short lived. Its only usefulness at present appears to be in pregnant women who have not had measles previously, in persons undergoing steroid therapy or in children with leukemia or similar blood dyscrasias—situations in which at-

tenuated measles vaccine is contraindicated. In the latter situation it would be particularly desirable to have data concerning the results of subsequent chance natural measles exposure in leukemic children, since inactivated vaccine does not prevent subsequent infection, and infection, with serious consequences, may still be theoretically possible in immunized leukemic children. Inactivated vaccine may also be used as a means of reducing the febrile reactions of subsequent Edmonston B vaccine by giving a single dose or a series of two doses at monthly intervals before the administration of the Edmonston B attenuated vaccine one month later.

When gamma globulin is administered in doses of 0.01 ml per pound of body weight simultaneously at a separate site, the reaction rate of Edmonston B vaccine is also reduced substantially. The Edmonston B vaccine now on the market has been used extensively without gamma globulin or without previous administration of killed vaccine. An appreciable proportion, perhaps 10 per cent, of recipients will have reactions of sufficient degree to warrant at least a telephone call and frequently a visit by a physician during the height of the reaction. While these reactions may be a nuisance and probably will preclude the widespread use of this vaccine alone in public clinics, they are of little real significance.

The recently released further attenuated virus, developed after additional passages of the Edmonston B strain at low temperature, would appear to resolve the problem of either giving multiple injections or risking the alternative, an excessive rate of reaction among recipients. In Table 4 data are presented which indicate the comparative reaction rates with the various alternate regimens listed above. Clearly, in terms of reaction rate alone, there is little difference as between the use of the further attenuated vaccine alone, the simultaneous administration of gamma globulin and Edmonston B, and the use of preparatory inoculation with inactivated vaccine followed in one month by the Edmonston B.

TABLE 4.—*Reactions Following Various Methods of Measles Immunization (Data from Krugman and Coworker[14])*

Type of Vaccine	*Symptoms (Per Cent)*		*Total Studied*
	Fever 103°+	*Rash*	
Edmonston B	30	50	175
Edm. B + Gamma Globulin (0.01-0.02ml/lb.)	15	12	854
Further Attenuated	15	5	569

An interesting facet of the exploration of attenuated measles virus is the complete absence of transmission to susceptibles exposed to immunized children and its very low infectivity, even when we administered it by aerosol directly into the respiratory tract at times of high prevalence of measles. This characteristic is in sharp contrast to the behavior of the natural disease. In addition, although highly infectious parenterally, the infectivity is not reduced by previous experience with inactivated vaccine, a finding similar to that in polioviruses. However, in contrast to polioviruses, small quantities of gamma globulin when administered before inoculation with attenuated measles vaccines prevent effective immunization. Maximal effectiveness of attenuated vaccine is not reached until the child is 12 months of age, presumably due to traces of maternal antibody still remaining in the infant, although at least 70 per cent of infants will respond at nine months of age. While it may be desirable in periods of high measles endemicity to administer attenuated measles vaccine as early as nine months of age, it should be noted that immunization is more effective (approaching 100 per cent) at and after one year of age.

Since this year, as previously, we have seen severe measles encephalitis follow natural exposure and the use of gamma globulin to modify the disease, we feel strongly that if exposure is known, measles should be prevented by the use of a larger preventive dose of gamma globulin, and then attenuated vaccine should be given at least two months later. If the precise day of exposure is known, an alternate approach is active immunization with attenuated vaccines, providing not more than two days has elapsed.

Although long-term data concerning resistance to infection following immunization with the attenuated viruses are not yet available, and will not become available for many years, antibody titrations following the administration of these vaccines are shown in Chart 1. While antibody titers following the administration of the further attenuated vaccine may not appear to persist at levels as high as in the case with Edmonston B or natural measles, they far exceed that necessary to prevent clinical measles, and failures in protection with either vaccine have been rare. Even if complete decay of circulating antibody should occur, it is likely that recall of immunity will be rapid and

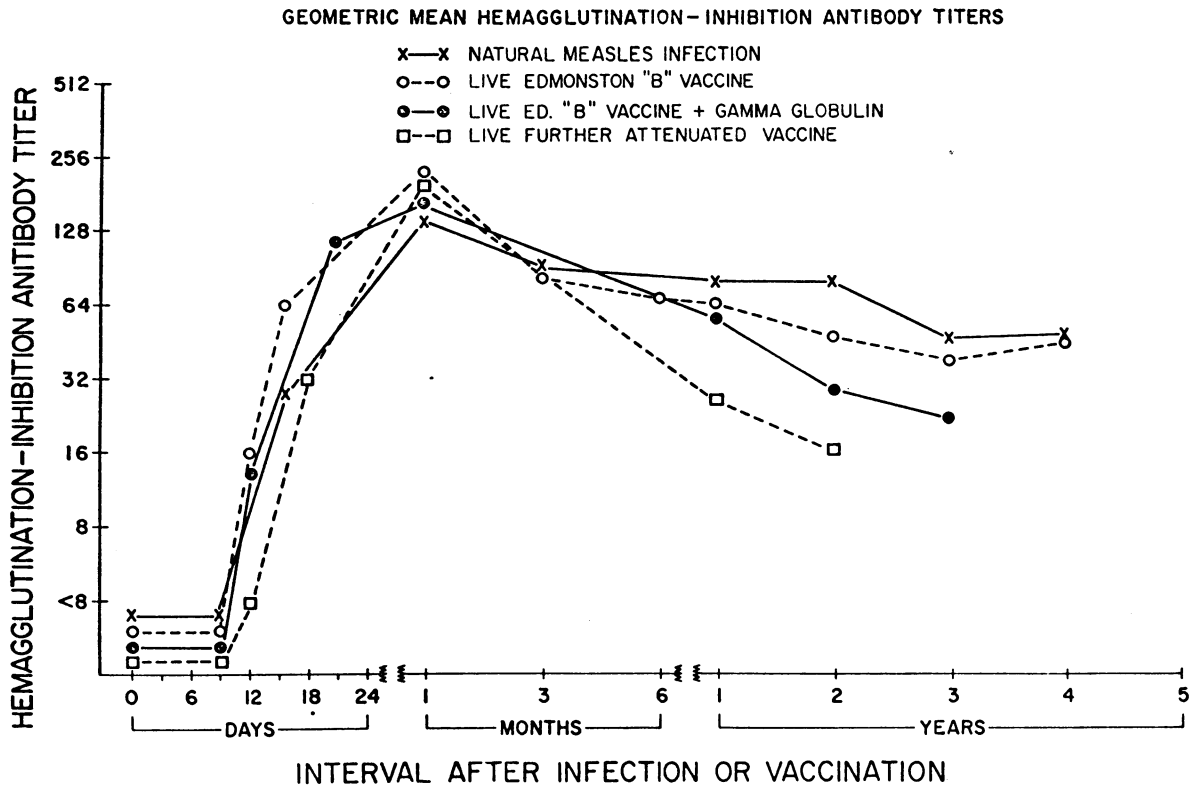

Chart 1.—Measles Antibody Response and Persistence Following Natural Infection and Vaccination. (From Comparison of the Pattern and Persistenc of the HI Antibody Response Following Natural Infection and Vaccination in Krugman, S., and Ward, R., Infectious Disease of Children, 3rd Ed., C. V. Mosby Co., 1964, page 136.)

clinical symptoms absent. In any event it seems apparent that any of these attenuated vaccine routines, if followed in infants one year of age and older, will be associated with extremely long-lasting if not permanent immunity to this disease.

No infectious disease has been brought under as prompt and effective control as has poliomyelitis. In 1964 only 121 cases, 94 of which were paralytic, were reported in the United States.[18] This incidence is less than one-fourth that of 1963, and only a single case has been reported thus far (March 20) in 1965.[19] Similar trends are seen for California in Table 5. It is of considerable importance that the usual seasonal increase in late summer and autumn was absent for the first time in 1964, suggesting either virtual absence of susceptible persons in the American community (a fact not borne out by numerous surveys) or profound interference with the transmission of this virus among infants and children, the well demonstrated reservoir of infection.

While it is possible that the striking decline in incidence from 1955 through 1961 was in part due to secular trends, changes in reporting and greater accuracy of diagnosis, over 400 million doses of inactivated poliovirus vaccine were administered during this period and without question this vaccine was mainly responsible for this decrease. Since 1961 more than 100 million doses of each of the three types of oral poliomyelitis vaccines have been distributed and more recently a considerably smaller quantity of oral trivalent vaccine.

The continued and accelerated decline during the last three years seems properly credited to the extensive use of oral vaccine. This decline in incidence occurred with decreasing use of inactivated

TABLE 5.—*Paralytic poliomyelitis, State of California, 1955-1964 (Data from California State Department of Public Health)*

Year Reported	*Total Patients*	*Rate Per 100,000*	*Deaths*
1955	1292	9.9	36
1956	1283	9.4	53
1957	291	2.1	19
1958	232	1.6	13
1959	408	2.7	20
1960	340	2.1	23
1961	75	0.5	4
1962	76	0.4	5
1963	15	0.08	1
1964	3	0.02	1

vaccine, the continued observation that approximately 20 per cent of paralytic disease reported occurred among persons who had received three or more doses of inactivated vaccine and occasional outbreaks of paralytic disease in communities relatively well immunized with inactivated vaccine.

Problems associated with the development of these poliomyelitis vaccines have been well documented[13] and require little discussion here. These include, with inactivated vaccine, difficulty in establishing adequate safety standards, with resultant paralytic disease among recipients in 1955.[15] In 1960 a relatively recently recognized virus, SV-40, was discovered in the vaccine originating from the tissue culture system, and this necessitated changes in safety testing to insure its absence. This relatively formalin-resistant virus is capable, in young rodents, of producing various malignant tumors and of transformation of human cells in tissue culture, although it must again be emphasized that no harmful effects have been noted in man. Problems of decreased immunologic potency, particularly of the type 3 component and subsequently the loss of pertussis antigenicity in attempts to combine diphtheria-pertussis-tetanus (DPT) and inactivated poliomyelitis vaccine further complicated application of this vaccine to prevention of disease.

During 1962 and subsequently, detailed records of cases of paralytic disease resembling poliomyelitis and occurring after administration of oral vaccine have been evaluated by special advisory committees to the Surgeon General of the Public Health Service. The most recent report[22] indicates that while it is not possible to prove that any individual case was caused by the vaccine, epidemiological evidence suggests that at least some cases were.

The maximum risk based on inclusion of all such possible disease associations appears to be substantially less than 1 per million doses administered, so it is not surprising that similar incidents were not seen with the Los Angeles County mass program of over seven million doses in 1962-63.[27]

Rapidly declining rates of poliomyelitis in this country during the last few years, the apparent minimal risk involved and the substantial effectiveness and both practical and theoretical advantages of oral vaccine suggest that certain changes may be desirable. During the past 18 months it has been apparent that adults remaining within the borders of the United States now have a negligible risk of naturally-acquired disease. Since the likelihood of vaccine-associated illness appears somewhat greater among adults than among young children, recent experience would suggest that widespread routine immunization of adults is no longer indicated or desirable. However, if these persons are in the armed forces, live in areas where poliomyelitis cases are occurring or travel outside this country, immunization should be provided, using oral vaccines.

The cases of two patients with paralytic poliomyelitis who were treated at Los Angeles County General Hospital in 1964 emphasize two extremely important points in the prevention of this disease. The first, an infant from Tiajuana, Mexico, with type 1 paralytic disease, indicates our proximity to areas with continued prevalence of endemic infection, and the need for immunization for persons traveling even to relatively nearby areas. The other, a fatal case of type 3 infection, occurred in an unimmunized infant residing in Los Angeles. Since four million infants are born each year in this country, attempts to reach and immunize these infants in spite of almost a certainty of increasing public apathy as the incidence of poliomyelitis decreases, presents a challenge to physician and health officer alike.

Active immunization against viral respiratory disease, utilizing both active and inactivated vaccines, has been studied for many years. Initial studies in this country with influenza[2,8] utilized active virus and this has been the principal direction of Russian investigation[28] and of limited studies in Britain.[17] Attention in this country and elsewhere has been directed toward formalin-inactivated vaccines, with or without adjuvants. A recently developed adjuvant[9] utilizing a metabolizable emulsified peanut oil has shown considerable promise in providing sustained response without the disadvantages of mineral oil adjuvants. Although the antigenic variation of influenza viruses is well known, the inclusion of recently isolated strains, with a suitable adjuvant, may provide a more dependable and effective agent in the future.

While the demonstrated potential of some adenoviruses to bring about tumors in rats[10,25] has temporarily slowed use of this antigen in man, it seems likely that this characteristic is associated with SV-40, a derivative of this virus, or similar agents present in the tissue culture system follow-

ing isolation of these viruses. Present efforts involve extension of earlier studies, utilizing the ability of active adenoviruses, when administered by mouth, to establish silent infections in the intestinal tract with excellent immunologic response. Thus far, two adenoviruses, types 4 and 7, have been shown to infect simultaneously and without discernible symptoms in the recipient.[4] Further studies can be expected to include trials of inactivated adenovirus vaccines, using fractions of as well as complete virus particles, and with and without adjuvants. In addition, trials of various carefully selected active or live adenoviruses known to be free of oncogenic potential can be expected to be continued.

Other respiratory viruses currently under investigation in various laboratories include the reoviruses, parainfluenza viruses and respiratory syncytial virus, although not enough information has become available as yet to assess the likelihood of success in the prevention of these infections by either inactivated or attenuated viral antigens. In addition, more information is needed concerning the relative importance of these agents as well as the large number of rhinoviruses which also have been associated with respiratory illness. It is apparent also that additional ecologic information concerning these agents is needed, and also more information on their potential role in the pathogenesis of some bacterial diseases.

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